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Cannabinoids and the gut: New developments and emerging concepts

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ABSTRACT

Cannabis has been used to treat gastrointestinal (GI) conditions that range from enteric infections and inflammatory conditions to disorders of motility, emesis and abdominal pain. The mechanistic basis of these treatments emerged after the discovery of Δ^9 -tetrahydrocannabinol as the major constituent of *Cannabis*. Further progress was made when the receptors for Δ^9 -tetrahydrocannabinol were identified as part of an endocannabinoid system, that consists of specific cannabinoid receptors, endogenous ligands and their biosynthetic and degradative enzymes. Anatomical, physiological and pharmacological studies have shown that the endocannabinoid system is widely distributed throughout the gut, with regional variation and organ-specific actions. It is involved in the regulation of food intake, nausea and emesis, gastric secretion and gastroprotection, GI motility, ion transport, visceral sensation, intestinal inflammation and cell proliferation in the gut. Cellular targets have been defined that include the enteric nervous system, epithelial and immune cells. Molecular targets of the endocannabinoid system include, in addition to the cannabinoid receptors, transient receptor potential vanilloid 1 receptors, peroxisome proliferator-activated receptor alpha receptors and the orphan G-protein coupled receptors, GPR55 and GPR119. Pharmacological agents that act on these targets have been shown in preclinical models to have therapeutic potential. Here, we discuss cannabinoid receptors and their localization in the gut, the proteins involved in endocannabinoid synthesis and degradation and the presence of endocannabinoids in the gut in health and disease. We focus on the pharmacological actions of cannabinoids in relation to GI disorders, highlighting recent data on genetic mutations in the endocannabinoid system in GI disease.

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Abbreviations: 2-AG, 2-arachidonoyl glycerol; AE, acylethanolamide; ACEA, *N*-(2-chloroethyl)5,8,11,14-eicosatetraenamide; ACh, acetylcholine; AP, area postrema; CB, cannabinoid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; CBDA, cannabidiolic acid; CBG, cannabigerol; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; DAGL, diacylglycerol lipase; DNBS, dinitrobenzene sulphonic acid; ENS, enteric nervous system; FAAH, fatty acid amide hydroxylase; GI, gastrointestinal; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; MGL, monoacylglycerol lipase; NADA, *N*-arachidonoyldopamine; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-selective phospholipase D; NGF, nerve growth factor; NO, nitric oxide; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; SP, substance P; THC, Δ^9 -THC, Δ^9 -THCA, Δ^9 -tetrahydrocannabinolic acid; Δ^9 -THCV, Δ^9 -tetrahydrocannabivarin; TNBS, trinitrobenzene sulphonic acid; TNF- α , tumour necrosis factor- α ; TRPV1, transient receptor potential vanilloid 1; VIP, vasoactive intestinal peptide.

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1. Introduction

Disorders of the gastrointestinal (GI) tract have been treated with herbal and plant-based remedies for centuries (Di Carlo & Izzo, 2003; Comar & Kirby, 2005). Prominent amongst these therapeutics are preparations derived from the marijuana plant *Cannabis* sp. (Di Carlo & Izzo, 2003). *Cannabis* has been used to treat a variety of GI conditions that range from enteric infections and inflammatory conditions, including inflammatory bowel disease (IBD) to disorders of motility, emesis and abdominal pain (Grinspoon & Bakalar, 1993; Izzo & Coutts, 2005). The mechanistic basis of these treatments gradually emerged after the discovery of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) as the major psychoactive constituent of *Cannabis*. Even before a specific receptor for Δ^9 -THC was cloned in 1990, progress had been made in identifying the site and mechanism of action of THC in the GI tract (Pertwee, 2001; Izzo & Coutts, 2005). For example, Gill et al. (1970) and then Roth (Roth, 1978) demonstrated that Δ^9 -THC inhibited cholinergic contractions of the ileum evoked by electrical stimulation of enteric nerves. Since this occurred in the absence of an effect on contractions produced by acetylcholine, it implied a presynaptic or prejunctional locus of action on acetylcholine release. These observations were confirmed and extended using isolated intestinal preparations and in whole animal studies. Cannabinoids (CBs) inhibit peristalsis and GI motility throughout the gut (Pertwee, 2001; Coutts & Izzo, 2004). Whilst these findings helped explain some of the therapeutic properties of *Cannabis*, they did not provide adequate explanations for the anti-inflammatory, anti-emetic and anti-secretory properties of *Cannabis*, as well as more recently described anti-proliferative actions.

After the discovery and cloning of the CB₁ and CB₂ receptors in 1990 and 1993, respectively (Matsuda et al., 1990; Munro et al., 1993), there was a renewed interest in the cannabinoid system. This led to the identification of endogenous cannabinoid ligands, anandamide and 2-arachidonylglycerol (2-AG, Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995) and the development of the concept of the endocannabinoid system (Di Marzo & Fontana, 1995). After these important discoveries, pharmacological, biochemical and molecular tools became widely available for investigations into the endocannabinoid system in the GI tract. This has led to considerable progress in describing the sites and mechanisms of actions of CBs in the gut, as described below. Much remains to be determined, but most of the actions of *Cannabis* and its derivatives can be at least partially explained.

One very significant development has been the identification of the biosynthetic and metabolic (degradative) pathways for the endocannabinoids (Piomelli, 2003; Di Marzo, 2009; Pertwee, 2009). Pharmacological tools have been discovered that, in particular, inhibit the enzymes responsible for the degradation of endocannabinoids. This allows for the manipulation of endocannabinoid levels in the gut, which has significant functional consequences and confirms the physiological and pathophysiological importance of the endocannabinoid system in the GI tract. In recent years, it has become clear that the endocannabinoids are part of a larger family of lipid mediators synthesized from common precursors, which act on both CB and other receptors, before having their actions terminated through common degradation pathways. In this focused review, we shall describe the endocannabinoid system in the gut, the pharmacological actions of CBs and recent developments in the therapeutic targets of the endocannabinoid system in the GI tract.

2. Cannabinoid targets and their localization in the gut

CBs by definition act at CB receptors. However, even from early studies it became clear that other receptor systems were involved in the actions of these pleiotropic molecules. The major receptors for CBs and their localization in the gut are described below.

2.1. Cannabinoid receptors

CB₁ and CB₂ receptors are the classical cognate receptors for all types of CB agonist – endocannabinoids, phytocannabinoids and synthetic CBs (Pertwee, 2009). Whilst there are examples of non-CB₁/CB₂ actions of CBs, there are no other molecularly-characterized CB receptors.

CB receptors have a distinct distribution in the GI tract, being largely distributed in the enteric nervous system (ENS, Duncan et al., 2005). Both CB₁ and CB₂ receptors are found by immunohistochemistry on enteric neurons, nerve fibres and terminals in the ENS. The CB₁ receptor is found on nerve fibres throughout the wall of the gut, but with the highest density in the two ganglionated plexuses, the myenteric and submucosal plexus, of the ENS (Duncan et al., 2005; Wright et al., 2008). Enteric ganglia consist of motor neurons, interneurons and intrinsic primary afferent neurons; CB₁ and CB₂ receptors appear to be localized on all of the functional classes of enteric neurons. Double-labelling immunohistochemistry of CB₁ receptor in neurons expressing choline-acetyltransferase, calretinin and substance P suggests that it is present on excitatory motor neurons (Kulkarni-Narla & Brown, 2000; Coutts et al., 2002), some classes of interneurons and intrinsic primary afferent neurons. The presence of CB₁ receptors on interneurons is also suggested by electrophysiological studies using multi-chambered organ baths (Yucec et al., 2007). Neither CB₁ nor CB₂ receptors are found on inhibitory motor neurons containing nitric oxide synthase (Kulkarni-Narla & Brown, 2000; Coutts et al., 2002; Storr et al., 2004; Duncan et al., 2008a). In rodents, immunoreactivity for the calcium binding protein calbindin is a marker for intrinsic primary afferent neurons. The CB₁ receptor is colocalized with calbindin (Coutts et al., 2002), suggesting that the CB₁ receptor is present on intrinsic primary afferent neurons. The presence of message for these receptors in the ENS was confirmed by in situ hybridization and reverse transcription polymerase chain reaction (Buckley et al., 1998; Storr et al., 2002).

Apart from the ENS, the pattern of cannabinoid receptor expression has not been fully elucidated in any species. There is a report of CB₁ receptors on the normal and inflamed human colonic epithelium, as well as in a number of colonic epithelial cell lines (Wright et al., 2005; Marqu ez et al., 2009), however, CB₁ receptor expression was not observed in the duodenal epithelium in controls or patients with celiac disease (D'Argenio et al., 2007). CB₁ receptors were also shown on parietal cells of the human stomach by immunohistochemistry and *in situ* hybridization (Pazos et al., 2008). CB₂ receptors appear to be present in the normal murine colonic epithelium, but not to any extent in the rat and human (Wright et al., 2005; Rousseaux et al., 2007; Marqu ez et al., 2009). However, there is an induction of CB₂ receptor immunoreactivity in the mouse and the rat GI epithelium after treatment with probiotic bacteria (Rousseaux et al., 2007) and in sections of the colon in patients with IBD (Wright et al., 2005; Marqu ez et al., 2009). Receptor binding studies revealed a distinct distribution of specific CB binding in the outer regions of Peyer's patches of the rat ileum (Lynn & Herkenham, 1994). These have not been followed up with immunohistochemical studies. In

contrast, *in situ* hybridization for CB₁ and CB₂ mRNA, albeit in developing animals, produced no obvious signal in the gut immune compartments (Buckley et al., 1998). In human colon, macrophages and plasma cells are immunoreactive for both CB₁ and CB₂ receptors (Wright et al., 2005). The distribution of CB receptors in the GI tract needs further systematic study in both the healthy gut and in pathophysiological states where CBs may be implicated in disease pathogenesis.

2.2. Transient receptor potential vanilloid 1 (TRPV1)

The endocannabinoid anandamide (arachidonylethanolamide) and the acylethanolamide (AE) oleoylethanolamide (OEA) are natural ligands of the TRPV1 receptor (Starowicz et al., 2007), best known as the receptor for the pungent principle of chilli peppers, capsaicin (Holzer, 2008a). The pharmacology is complex, with full or partial agonism depending on the level of receptor expression (Ross, 2003). TRPV1 receptors are expressed throughout the GI tract on the terminals of extrinsic primary afferent fibres and in some immune cells in the mucosa. The highest density of TRPV1 immunoreactivity is in the myenteric plexus and the interganglionic nerve fibre tracts (Ward et al., 2003). Varicose TRPV1 immunoreactive nerves are also observed running within the muscle layers and along the blood vessels of the submucosa (Ward et al., 2003; MacNaughton et al., 2004). TRPV1-like nerves and some immunoreactive cells are observed within the mucosa (Holzer, 2008b). Most of the TRPV1 immunoreactive nerves are of spinal afferent origin, but in the proximal gut there are some vagal afferents that express TRPV1 (Ward et al., 2003).

2.3. Peroxisome proliferator-activated receptor alpha (PPAR α)

Oleoylethanolamide and palmitoylethanolamide (PEA) are naturally occurring AEs, chemically related to anandamide. These molecules all share enzymes involved in their biosynthesis (i.e., NAPE-PLD: *N*-acyl-phosphatidylethanolamine [NAPE]-selective phospholipase D [PLD]), and enzymatic degradation (i.e., FAAH: fatty acid amide hydrolase) (Borrelli & Izzo, 2009; Godlewski et al., 2009). However, in contrast to anandamide, OEA and PEA do not activate CB receptors and hence cannot be considered endocannabinoids. Rather, OEA and, to a lesser extent, PEA activate PPAR α . PPAR α is a ligand-activated transcription factor, but its activation has also been shown to induce rapid, cellular changes that do not require transcription (so called non-genomic effects) including the induction of satiety (OEA) and reduction of inflammation (PEA) (Lo Verme et al., 2005; O'Sullivan, 2007; Thabuis et al., 2008). Both OEA and PEA have a number of actions in the GI tract that will be discussed below. It should be noted that THC and possibly anandamide are also PPAR α agonists (Brown, 2007; O'Sullivan, 2007).

PPAR α immunoreactivity is highly expressed in enterocytes in the small intestine and was recently reported in enteric neurons of the myenteric and submucosal plexuses throughout the GI tract (Thabuis et al., 2008; Cluny et al., 2009). PPAR α is also found on vagal afferent fibres in the GI tract where it mediates the effects of OEA on satiety. mRNA for PPAR α is found in all regions of the rat gut from the oesophagus to the colon and is mirrored by PPAR α protein expression (Wang et al., 2005).

2.4. Orphan G-protein coupled receptors: GPR55 and GPR119

The "orphan" G-protein-coupled receptors GPR55 and GPR119 have recently been proposed as molecular target of some AEs.

GPR119 is expressed on enteroendocrine L cells of the GI tract, where it regulates the release of the anti-diabetic peptide glucagon-like peptide-1 (Chu et al., 2008; Overton et al., 2008; Lauffer et al., 2009). It is also found on pancreatic β cells in the islets of Langerhans. Oleoylethanolamide is one of the most potent ligands for this receptor, which is not activated by anandamide and only weakly by PEA (Godlewski et al., 2009).

GPR55 was identified as a novel CB receptor, though showing virtually no apparent homology to either of the classical CB receptors (Godlewski et al., 2009). The natural ligands probably include PEA, anandamide, and other CBs, but OEA is a weak agonist. It is also a receptor for lysophosphatidylinositol (Oka et al., 2009). mRNA for GPR55 is found in the GI tract (Ryberg et al., 2007), with the highest expression in the small intestine and minimal expression in the stomach and colon, but the distribution of this receptor has not yet been studied in any detail.

3. Proteins involved in endocannabinoid synthesis and degradation in the gut

Biosynthetic and degradative pathways have been identified for anandamide and 2-AG. Unlike classical neurotransmitters, or many other intercellular signalling molecules, which are stored in vesicles before release, anandamide and 2-AG are synthesized 'on demand' from the remodelling of membrane lipids (Piomelli et al., 2000; Di Marzo, 2008a).

Anandamide biosynthesis is usually triggered by an elevation of intracellular calcium from influx through calcium channels or by stimulation of intracellular stores (Solinas et al., 2008; Di Marzo, 2009; Petrosino et al., 2009). A calcium-dependent acyl-transferase catalyzes the formation of *N*-arachidonoyl phosphatidylethanolamine from arachidonic acid and phosphatidylethanolamine. Anandamide is then formed from the one-step hydrolysis catalyzed by NAPE-PLD. However, since NAPE-PLD knockout mice display normal levels of anandamide in the brain and some other tissues (Leung et al., 2006), additional pathways for its formation must exist. Three additional pathways have been demonstrated that catalyze the formation of anandamide from *N*-arachidonoyl phosphatidylethanolamine. First, phospholipase C catalyzes an intermediate phospho-anandamide which is then dephosphorylated by a phosphatase (Liu et al., 2006); second, α/β -hydrolase 4 leads to the formation of a glycerol-phospho-anandamide which is then hydrolyzed further by phosphodiesterase to form anandamide (Simon & Cravatt, 2006); and third, Sun et al. (2004) showed that anandamide could be formed from the conversion of *N*-arachidonoyl phosphatidylethanolamine to a 2-lyso intermediate by soluble phospholipase A₂ followed by cleavage by lyso-phospholipase D. Currently, it is not clear which of these pathways is used for the formation of anandamide in the GI tract, nor whether this changes under pathophysiological conditions.

2-AG formation involves the sequential activation of phospholipase C and diacylglycerol lipase (DAGL) on the precursors phosphatidic acid and phosphatidylinositol. Two DAGLs (α and β) have been cloned and identified as responsible for the formation of 2-AG (Bisogno et al., 2003), but these have yet to be directly demonstrated in the GI tract or specifically in the ENS.

Inactivation of anandamide and 2-AG occurs intracellularly. The dominant enzyme responsible for the hydrolysis of anandamide (and other *N*-acyl-ethanolamines) is fatty acid amide hydrolase (FAAH). FAAH is a membrane-bound hydrolase enzyme, which shows considerable, but not complete, overlap with CB₁ receptor in rat brain (Ahn et al., 2008); FAAH inactivates both anandamide and 2-AG. However, more recent evidence including studies using FAAH-deficient mice confirm its importance for anandamide (Ahn et al., 2008), but suggest that it has a lesser role in 2-AG signalling *in vivo*. FAAH mRNA and protein have been reported in both rat stomach and rat and mouse small and large intestine (Katayama et al., 1997; Izzo et al., 2001b; Capasso et al., 2005). FAAH is localized to cell bodies in the myenteric plexus (Duncan et al., 2005) but which classes of enteric neuron that express FAAH are not known. Inhibitors of FAAH are highly effective modulators of GI motility and inflammation, highlighting the importance of its substrates in physiology and pathophysiology of the gut, as will be discussed below.

Monoacylglycerol lipase (MGL) is the principal 2-AG hydrolase. MGL is found in the brain and also displays overlap with the central CB₁ receptors (Ahn et al., 2008). Recently, MGL was localized in the GI tract (Duncan et al., 2008b). MGL mRNA and protein are distributed in the muscle and mucosal layers of the ileum and in the duodenum, proximal colon, and distal colon. MGL expression was found in most nerve cell bodies and also in nerve fibres of the ENS, but like the CB receptors, it was not found in nitric oxide synthase containing neurons. MGL was also present in the epithelium and was highly expressed in the small intestine. Enzyme activity levels were highest in the duodenum and decreased along the gut, with lowest levels in the distal colon. The MGL inhibitor URB602 inhibited whole gut transit in mice through a CB₁ receptor-dependent mechanism (Duncan et al., 2008b). Two additional 2-AG hydrolases have been identified, α/β -hydrolase 6 and 12 (Ahn et al., 2008). The subcellular distribution of these enzymes and MGL are distinct, suggesting that they may regulate different pools of 2-AG in the cell. It is not known if these novel enzymes contribute to the hydrolysis of 2-AG in the GI tract.

Finally, both anandamide and 2-AG are reported to be taken up into cells by facilitated diffusion via a protein transporter in the cell membrane (Di Marzo et al., 1994). The putative endocannabinoid membrane transporter has been characterized, but never cloned, and there is evidence for and against its existence (Glaser et al., 2005). However, drugs that have activity against this transporter are effective in the GI tract as discussed below.

4. Endocannabinoids and their presence in the gut

Because the cellular localization of the biosynthetic enzymes for endocannabinoid production is not well understood, the cellular sources of endocannabinoids and the specific stimuli for endocannabinoid synthesis in the gut remain to be fully determined (Izzo & Camilleri, 2008). There are regional variations in the levels of endocannabinoids in the gut, with 2-AG being higher in the ileum than the colon, and anandamide being considerably higher in the colon than the ileum (Izzo et al., 2001b; Pinto et al., 2002). Levels of anandamide are elevated in rat and mouse models of colitis and in mucosal biopsy samples from patients with IBD (D'Argenio et al., 2006). There were differences in the distribution of these increases between human and animal samples. The increases observed in the mucosa of patients with ulcerative colitis were not observed in rat tissues, which displayed increases in the muscle and submucosal layers of the gut. These may be due to species differences or to the forms of colitis. By contrast, anandamide levels were not altered in croton oil-induced ileitis (Izzo et al., 2001b), and neither were levels of 2-AG in rat and mouse models of colitis (D'Argenio et al., 2006).

The state of satiety of an animal alters the levels of anandamide in the gut. After a 24-h fast, rats show increased levels in the small intestine, but not the stomach (Gómez et al., 2002). The mechanism of food deprivation-induced alterations in *N*-acylethanolamides in the intestine has recently been shown to be due to a remodelling of the precursor *N*-acylphosphatidylethanolamine with amide-linked oleate and arachidonate (Petersen et al., 2006). The levels of endocannabinoids in the GI tract are also altered in pathophysiological conditions and after treatment with certain drugs. For example, in coeliac disease, levels of anandamide measured in mucosal biopsies are increased, but return to normal after treatment with a gluten-free diet (D'Argenio et al., 2007). In contrast, 2-AG levels were unchanged in this condition. This pattern is different to what is observed in the mucosa in cancer. In colonic biopsies from patients with polyps or colorectal cancer there are highly significant increases in both 2-AG and anandamide levels (Ligresti et al., 2003), in the absence of any changes in FAAH expression, suggesting that there was enhanced biosynthesis. Finally, in diverticulosis, the colonic levels of anandamide are once again increased, but 2-AG is actually below control levels (Guagnini et al., 2006b).

Differential effects on endocannabinoid levels are seen in animal studies. Treatment of rats with methotrexate, which to some extent models the histological features of coeliac disease, produced modest changes in the levels of anandamide, but substantial increases in 2-AG in both the mucosa and muscle layers of the jejunum (D'Argenio et al., 2007). These results again highlight potentially relevant human vs. animal differences in the production of endocannabinoids in the gut. The consequences of elevated or reduced CB levels have yet to be fully defined. It may be compensatory in some cases, for example, anti-inflammatory or orexigenic actions of anandamide, but this may not always be the case. Much more work is needed to relate the activity or expression of biosynthetic pathways, the cellular sites of endocannabinoid production and the conditions under which changes are observed (disease, drugs, etc.) to their actions. This level of sophistication is possible with comprehensive lipidomic and proteomic approaches to GI tissues and will be very rewarding once accomplished.

There is a number of other putative endocannabinoids, including *N*-arachidonoyldopamine (NADA), an agonist at both CB₁ and TRPV1 receptors (Huang et al., 2002; O'Sullivan et al., 2004; Ralevic, 2003), and virodhamine, a potential endogenous antagonist of the CB₁ receptor (Porter et al., 2002). Neither these, nor many other potential endocannabinoid signalling molecules have been adequately characterized or measured in the GI tract. A summary of the adaptive changes of the endocannabinoid system that occurs in the GI tract is given in Tables 1 and 2.

5. Physiological and pharmacological actions

5.1. Food intake and feeding behaviour

Cannabis is well known to cause the “munchies” or a craving for particularly high fat foods. An explanation for this phenomenon is that the endogenous CB system regulates energy balance and food intake at several functional levels, both in the brain and the periphery, including the GI tract (Bellocchio et al., 2008). In a number of species, including humans, CB receptor agonists including Δ^9 -THC increase food intake and promote body weight gain via CB₁ receptor activation (Kunos, 2007; Matias and Di Marzo, 2007). Conversely, selective CB₁ receptor antagonists reduce food intake and body weight in animals and humans (Di Marzo, 2008b). Despite success in clinical trials, rimonabant, the first available member of this class of drugs, was withdrawn from the European market in October 2008, because of an increased risk of depression. However, it was an effective weight loss agent (Van Gaal et al., 2005; Pi-Sunyer et al., 2006; Van Gaal et al., 2008), and provided proof of the translational therapeutic concept in humans that the endocannabinoid system was important in energy balance regulation.

The intestinal endocannabinoid system undergoes adaptive changes in response to diet. Food deprivation increases anandamide levels, as noted above. In concert with these changes there is an up-regulation of CB₁ receptor expression in vagal afferent neurons that project to the GI tract (Gómez et al., 2002; Burdyga et al., 2004). In fed rats, low levels of CB₁ message and protein expression are observed in the nodose ganglion, but in fasted rats, message and protein expression are increased substantially and the distribution of the receptor is found throughout the ganglion, notably in the caudal pole that contains the vagal afferent neurons projecting to the GI tract (Burdyga et al., 2004). These findings suggest that CB₁ receptors, located on vagal afferent neurons, may be involved in CB-induced modulation of appetite and that anandamide might act as a “hunger signal” in the intestine (for further discussion see Storr & Sharkey, 2007 and Borrelli & Izzo, 2009).

If the GI endocannabinoid system is involved in energy balance regulation, are there changes during the development of obesity? Recently, Paulino et al. (2009) showed that expression of CB₁ receptor message was up-regulated in the nodose ganglia of obesity-prone rats fed a high fat diet compared to those resistant to diet-induced obesity.

Table 1

Summary of adaptive changes of the endogenous CB system (endocannabinoid levels, CB receptors and protein involved in endocannabinoid biosynthesis and degradation) in experimental pathophysiological states.

Experimental/condition	Induced by	Animal species/region of gut	Endocannabinoid changes	Receptor changes	Enzyme changes	References
Obesity	High fat diet	Mouse, stomach	Decreased levels of anandamide (but not 2-AG)	Decreased CB ₁ receptor mRNA expression	Increased NAPE-PLD mRNA expression; decreased FAAH mRNA expression	Aviello et al., 2008a; Di Marzo et al., 2008
Obesity	High fat diet	Mouse, small intestine	Decreased levels of anandamide; increased levels of 2-AG	ND	ND	Izzo et al., 2009b
Obesity	Genetic model (Zucker rats)	Rat, duodenum	Increased levels of both anandamide and 2-AG	ND	ND	Izzo et al. 2009b
Ileitis	LPS	Rat, small intestine	ND	No changes in CB ₂ receptor mRNA expression	ND	Duncan et al., 2008b
Ileitis	Croton oil	Mouse, small intestine	No changes in endocannabinoid levels	Increased CB ₁ receptor protein expression	Increased FAAH activity	Izzo et al., 2001b
Secretory diarrhoea	Cholera toxin	Mouse, small intestine	Increased levels of anandamide (but not 2-AG)	Increased CB ₁ receptor mRNA expression	No changes in FAAH mRNA	Izzo et al., 2003
Secretory diarrhoea	<i>C. difficile</i> toxin A	Rat, ileum	Increased levels of both anandamide and 2-AG	ND	ND	McVey et al., 2003
Ileus	Acetic acid	Mouse, small intestine	Increased levels of anandamide (but not 2-AG)	Increased neural density of CB ₁ receptor immunohistochemistry	No changes in FAAH activity	Mascolo et al., 2002
Colitis	DNBS	Mouse, colon	Increased levels of both anandamide and 2-AG	Increased number of CB ₁ receptor-expressing neurons	Decreased FAAH mRNA expression	Massa et al., 2004; Borrelli et al., 2009
Colitis	TNBS, oxazolone, DSS	Mouse, colon	ND	ND	Decreased FAAH mRNA expression	Storr et al., 2008
Colitis	Oil of mustard	Mouse, colon	ND	CB ₁ and CB ₂ receptor up-regulation (immunohistochemistry)	ND	Kimball et al., 2006
Aberrant crypt foci (preneoplastic lesions)	Azoxymethane	Mouse, colon	Increased levels of 2-AG (but not anandamide)	No changes in CB ₁ and CB ₂ receptor mRNA expression	No changes in FAAH mRNA expression	Izzo et al., 2008
Coeliac-like atrophy	Methotrexate	Rat, duodenum	Increased levels of both anandamide and 2-AG	ND	ND	D'Argenio et al., 2007

Abbreviations: 2-AG, 2-arachidonoyl glycerol; CB, cannabinoid; DNBS, dinitrobenzene sulphonic acid; DSS, dextran sulphate sodium; FAAH, fatty acid amide hydrolase; LPS, lipopolysaccharide; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-selective phospholipase D; TNBS, trinitrobenzene sulphonic acid; ND = not determined.

These data suggest that there is a greater capacity to transmit orexigenic signals from the periphery in animals prone to obesity. Moreover, Izzo et al. have shown that anandamide levels are up-regulated in the small intestine of mice fed a high fat diet and also in fasted obese Zucker rats (Izzo et al., 2009b; Izzo et al., 2009c). Striking changes in endocannabinoid levels were observed in the duodenum of obese Zucker rats under conditions of fasting and refeeding. Not only were basal levels of the endocannabinoids anandamide and 2-AG higher (about 2 fold for anandamide and 9 fold for 2-AG), but they increased substantially in the fasting state (about 4 fold in lean vs. 9 fold in the Zucker rats for anandamide and about 2 fold in lean vs. 4 fold in Zucker rats for 2-AG) and were still elevated after refeeding (Izzo et al., 2009b). Taken together these data illustrate the potential of gut-derived endocannabinoids to provide a strong orexigenic drive at peripheral receptors, sustaining or stimulating food intake in obesity. There are some caveats of this work related to the

disregulated leptin signalling in the Zucker rats, but even taking them into consideration, this study provides support to the idea that the endocannabinoid system of the GI tract plays a significant role in the maintenance of elevated body weight leading to obesity. Of course, if this is true, then peripherally restricted CB₁ receptor antagonists should be effective in limiting food intake and reducing body weight (see Kunos et al., 2009). Data supporting this idea were presented as long ago as 2002 (Gómez et al., 2002). Gómez et al. showed that centrally administered rimonabant did not block food intake, and both the orexigenic actions of CBs and anorexigenic effects of peripherally administered rimonabant were blocked after selective chemical vagal deafferentation. Recently, Lo Verme et al. (2009) developed a novel peripherally restricted CB₁ receptor antagonist (URB447) and showed that it was effective at suppressing food intake and reducing body weight in genetically obese *ob/ob* mice. Future development of these agents offers a promising therapeutic approach

Table 2

Summary of adaptive changes of the endogenous CB system (endocannabinoid levels, CB receptors and protein involved in endocannabinoid biosynthesis and degradation) in intestinal human pathologies.

Pathology	Region of the gut	Endocannabinoid changes	Receptor changes	Enzyme changes	References
Coeliac disease	Duodenum	Increased levels of anandamide and a trend towards an increase in 2-AG	Increased CB ₁ receptor immunofluorescence	ND	D'Argenio et al., 2007
Diverticulitis	Colon	Increased levels of anandamide, decreased levels of 2-AG	No changes in CB ₁ and CB ₂ receptor mRNA expression	ND	Guagnini et al., 2006b
Colorectal carcinoma	Colon	Increased levels of anandamide and 2-AG	Decreased CB ₁ receptor mRNA expression; increased CB ₂ receptor mRNA expression	No changes in FAAH expression	Ligresti et al., 2003; Cianchi et al., 2008
Ulcerative colitis	Colon	Increased levels of anandamide (but not 2-AG)	Increased CB ₁ and CB ₂ receptor expression	Increased DAGL α and MGL expression	Wright et al., 2005; D'Argenio et al., 2006; Marquez et al., 2009

Abbreviations: 2-AG, 2-arachidonoyl glycerol; CB, cannabinoid; DAGL α , diacylglycerol lipase α ; FAAH, fatty acid amide hydrolase; DAGL α , diacylglycerol lipase α ; MGL, monoacylglycerol lipase. ND, not determined.

to obesity and its complications, with less potential for unwanted central side-effects such as depression.

5.2. Nausea and emesis

Nausea and vomiting (emesis) are frequent and unpleasant symptoms of many diseases, as well as a side-effect of medications used in the treatment of pain (e.g. opiates), cancer (chemotherapeutics) and a variety of other conditions. CBs are effective anti-emetics (Tramèr et al., 2001), though not first-line therapeutics because of their central side-effects. However, CBs are also able to suppress nausea to some extent (Tramèr et al., 2001; Slatkin, 2007), which is a feature not shared by some highly effective anti-emetics such as the 5-HT₃ receptor antagonists. Curiously, in some people, chronic use of CBs results in a clinical condition known as CB hyperemesis, which can be relieved by hot showers (Allen et al., 2004; Wallace et al., 2007; Chang & Windish, 2009; Donnino et al., 2009; Sontineni et al., 2009). This paradoxical condition is a cyclic vomiting illness. Currently, there is a very limited understanding of the pathophysiology of CB hyperemesis, and no explanation for the efficacy of hot showers. This unusual condition awaits further analysis.

Emetic stimuli activate neurons in the dorsal vagal complex in the brainstem. The dorsal vagal complex consists of the area postrema (AP), dorsal motor nucleus of the vagus and nucleus of the solitary tract. The AP, also known as the chemoreceptor trigger zone, is a circumventricular structure located on the floor of the 4th ventricle. The AP receives input from vagal afferents in the gut and elsewhere, which it integrates with information sampled from the cerebrospinal fluid and blood that bathes this richly vascularised organ. The nucleus of the solitary tract receives vagal and spinal afferent inputs from the gut and integrates these with inputs from the AP to regulate the efferent outflow to the proximal GI tract from neurons of the dorsal motor nucleus of the vagus that initiate the motor programs of reverse peristalsis that leads to emesis. The central pathways of nausea are not well defined, but overlap to some extent with those of emesis (Hornby, 2001).

CBs inhibit emesis in animal models of acute cisplatin-, morphine- and radiation-induced emesis (Darmani, 2001a, Simoneau et al., 2001; Van Sickle et al., 2001, 2003, 2005; Darmani et al., 2007). Recently, these observations were extended to show that CBs were effective in cisplatin-induced delayed emesis (Ray et al., 2009), and in motion sickness (Cluny et al., 2008). Parker et al. (2009) showed that the FAAH inhibitor URB597 suppressed cisplatin- and nicotine-induced vomiting in the house musk shrew through CB₁ receptors.

A description of the endocannabinoid system in the dorsal vagal complex provided a neuroanatomical substrate to explain the mechanism of the anti-emetic action of CBs (Darmani, 2001b; Van Sickle et al., 2001, 2003, 2005; Sharkey et al., 2007). Not only are CB₁ receptors present in this region, but, surprisingly, so are CB₂ receptors (Van Sickle et al., 2005), which were previously thought to be located peripherally, and not in the central nervous system. These were discovered by examining the anti-emetic effects of raising local extracellular levels of endocannabinoids in the brainstem. It was found that not only were these responses reversed by a CB₁ receptor antagonist, they were also sensitive to CB₂ receptor antagonism. The brainstem CB₂ receptors are able to inhibit emesis when co-stimulated with CB₁ receptors by endocannabinoids capable of activating both receptors (Van Sickle et al., 2005).

As described above, endocannabinoids activate TRPV1 as well as CB₁ and CB₂ receptors. The TRPV1 receptor in the dorsal vagal complex is found in the AP and nucleus of the solitary tract on vagal afferent terminals (Patterson et al., 2003). Both NADA and anandamide reduce emesis through CB₁ or TRPV1 receptors or both (Sharkey et al., 2007). Their actions were attenuated by a CB₁ receptor antagonist, which was pro-emetic per se, and TRPV1 antagonists which were without emetic effects when administered alone. These observations suggest that agonists of CB₁, (CB₂) and TRPV1 receptors in the brainstem are involved in the control of emesis. Whilst there appears to be an endogenous

“tone” of CB₁ receptors, this does not seem to be the case for TRPV1 receptors. It should be noted that there are also synergistic interactions between CB₁ and 5-HT₃ receptors in attenuating cisplatin-induced acute emesis (Kwiatkowska et al., 2004; Wang et al., 2009), which may be potentially very useful if exploited clinically.

Investigating nausea in laboratory rodents is difficult because of the subjective nature of this sensation; however, behavioural models do exist. One such model is the conditioned taste avoidance test. A flavoured liquid is paired with a compound and subsequent avoidance of this flavour can reflect the potential of the compound to be noxious and induce illness. CB₁ receptor antagonists/inverse agonists including rimonabant (De Vry et al., 2004) and AM251 (McLaughlin et al., 2005) have been shown to induce conditioned taste avoidance in rats, suggesting that they have the potential to induce nausea. In contrast, Δ⁹-THC prevented the development of avoidance in the house musk shrew (Kwiatkowska & Parker, 2005). A more selective model of nausea is the taste reactivity test. When noxious compounds are paired with an intra-oral infusion of saccharin, rats display gaping and a reduction in the number of other hedonic (pleasure-seeking) behaviours (Parker, 2003; Parker et al., 2008). The CB₁ receptor antagonist/inverse agonist AM251 induced gaping in the taste reactivity test in rats (McLaughlin et al., 2005), suggestive of nausea, however, the neutral CB₁ receptor antagonist AM4113 did not demonstrate the potential to induce nausea in this paradigm (Sink et al., 2008). The FAAH inhibitor URB597 dose-dependently prevented rejection reactions and increased ingestive reactions in this model, showing it was reducing nausea (Cross-Mellor et al., 2007). These effects were abolished by AM251 showing that they were mediated by endogenously released endocannabinoids acting at CB₁ receptors.

Anticipatory nausea is commonly experienced in cancer chemotherapy, especially in patients whose anti-emetic therapy was unsuccessful. Modulation of the endocannabinoid system is also effective in animal models of anticipatory nausea: conditioned gaping or retching. Both Δ⁹-THC and URB597 suppress these responses in rats and shrews (Parker & Kemp, 2001; Parker et al., 2006; Rock et al., 2008). Consistent with clinical experience, the 5-HT₃ receptor antagonist ondansetron was ineffective in these paradigms.

Taken together it is apparent that there is considerable untapped therapeutic potential in utilizing the endocannabinoid system for the treatment of nausea and emesis by modulation of endogenous CBs with drugs that enhance the local production or interfere with the degradation of these molecules in the central nervous system.

5.3. Gastric secretion and gastroprotection

CBs decrease acid production in rodents through activation of CB₁ receptors (Adami et al., 2002, 2004; Coruzzi et al., 2006). The site of action is on vagal efferent pathways to the gastric mucosa and not on parietal cells. Indeed, CBs decrease acid secretion induced by 2-deoxy-D-glucose and pentagastrin (which increase acid secretion through the release of acetylcholine), but not histamine, which activates H₂ receptors on parietal cells to produce an increase in acid secretion (Adami et al., 2002). The recent findings of CB₁ receptors on human parietal cells (Pazos et al., 2008) point to species differences, as CBs may directly inhibit acid secretion in humans. However, in the absence of clinical evaluation this remains to be determined.

CB₁ receptor activation is protective in animal models of gastric ulcers induced by aspirin (Rutkowska & Fereniec-Gołbiewska, 2006), water immersion and restraint stress (Dembiński et al., 2006) and cold/restraint stress (Germanò et al., 2001). CB receptor antagonists administered alone aggravated gastric damage induced by water immersion and restraint stress (Dembiński et al., 2006) and stimulated gastric acid secretion in vitro (Borrelli, 2007). In addition, Naidu et al. have shown that FAAH-deficient mice as well as the FAAH inhibitor URB597 displayed a significant amelioration in the magnitude of gastric irritation caused by diclofenac in mice (Naidu et al., 2009). Collectively,

such findings suggest a pathophysiological involvement of the endogenous CB system – via CB₁ receptor activation – in gastroprotection. The data also indicate that FAAH inhibitors may represent novel adjuvant therapies for treating gastric irritation by providing gastroprotection and, potentially, antinociception when used in combination with traditional non-steroidal anti-inflammatory agents.

5.4. Lower oesophageal sphincter

The lower oesophageal sphincter is a specialized region of the oesophageal circular smooth muscle that allows the passage of a swallowed bolus to the stomach and prevents the reflux of gastric contents into the oesophagus (Farré & Sifrim, 2008). CB receptor agonists inhibited transient lower oesophageal sphincter relaxations in dogs and ferrets via CB₁ activation (Lehmann et al., 2002; Partosoedarso et al., 2003; Beaumont et al., 2009), the effect being associated, at least in the dog, with the inhibition of gastroesophageal reflux (Lehmann et al., 2002; Beaumont et al., 2009). This is in agreement with the observation that CB₁ receptor immunoreactivity is present in neurons within the dorsal vagal complex (i.e. the AP, nucleus of the solitary tract) and nodose ganglion (Lehmann et al., 2002; Partosoedarso et al., 2003).

In line with animal studies, Δ^9 -THC reduced the number of transient lower oesophageal sphincter relaxations and caused a non-significant reduction of acid reflux episodes in the first postprandial hour in healthy volunteers (Beaumont et al., 2009). In addition, lower oesophageal sphincter pressure and swallowing were significantly reduced by Δ^9 -THC. Central and peripheral vagal mechanisms are involved in these functional changes (Beaumont et al., 2009). An interesting study by Cui et al. (2007) extends the potential therapeutic benefits of CBs in gastroesophageal reflux disease. They demonstrated in a guinea pig model of gastroesophageal reflux that CB₂, but not CB₁, receptor activation was capable of reducing the microvascular leakage and bronchoconstriction observed by instillation of hydrochloric acid into the oesophagus. Taken together, these findings support the use of CB agonists or drugs that elevate the levels of endogenous CBs for the treatment of gastroesophageal reflux disease as an adjunct therapy with acid inhibition.

5.5. Gastrointestinal motility

5.5.1. Enteric transmission and peristalsis

It is generally accepted that CB receptor agonists act on prejunctional CB₁ receptors to reduce smooth muscle contractility in different regions of the GI tract in animals and humans (Izzo & Camilleri, 2008). The action is similar to the effect of μ -opioid receptor and α_2 -adrenoceptor agonists in intestinal tissues. Thus, CBs – via CB₁ receptor activation – have been shown to reduce electrically-induced contractions in the i) mouse (Mulè et al., 2007b) or rat stomach (Storr et al., 2002), ii) guinea pig (Pertwee et al., 1996; Izzo et al., 1998; Begg et al., 2002; Abalo et al., 2005) and human ileum (Crocì et al., 1998; Manara et al., 2002) and iii) human colon (Manara et al., 2002; Guagnini et al., 2006b; Hinds et al., 2006). Both the longitudinal and the circular muscles are generally responsive to the inhibitory action of CB receptor agonists. Although the mechanisms by which CB₁ receptor activation reduces contractility are mainly related to reduction of acetylcholine release from prejunctional nerves, other mechanisms, which are possibly limited to restricted parts of the gut and/or to some animal species, have been proposed. These include inhibition of non-adrenergic–non-cholinergic excitatory (Izzo et al., 1998; Mulè et al., 2007a) and inhibitory transmission (Storr et al., 2004), modulation of the purinergic system via the P2X receptors (Begg et al., 2002; Baldassano et al., 2009) and activation of CB₂ receptors (Mulè et al., 2007b) (see below). There is also evidence that anandamide and 2-AG may inhibit myogenic contractions of the human circular and longitudinal muscle through a CB-independent mechanism (Smid et al., 2007).

CBs inhibit peristaltic propulsion in isolated rodent intestinal segments, both in the small and in the large intestine (Heinemann

et al., 1999; Izzo et al., 2000a; Mancinelli et al., 2001; Yucece et al., 2007; Sibaev et al., 2009; Grider et al., 2009). This effect has been largely attributed to inhibition of the ascending contraction component (ascending interneurons and final motor neurons) as a result of CB₁ receptor-mediated inhibition of acetylcholine release (Yucece et al., 2007; Aviello et al., 2008b; Sibaev et al., 2009). However, recent evidence suggests that the anti-propulsive actions are likely the result from reduction of all the components of the peristaltic reflex, at least in the rat colon. Indeed Grider et al. have recently shown that CBs inhibit i) the ascending contraction and concomitant substance P release, ii) the descending relaxation and concomitant vasoactive intestinal peptide (VIP) release and iii) the sensory limb and concomitant calcitonin gene-related peptide (CGRP) release via CB₁ receptor activation (Grider et al., 2009) (see Fig. 1).

To date, there is less evidence that CB₂ receptors are involved in the control of normal motility. Storr et al. (2002) showed that the CB₂ receptor antagonist AM630 blocked the anandamide-induced inhibition of electrical field stimulated contractions and non-adrenergic non-cholinergic relaxations of the rat gastric fundus. Furthermore, AM630 alone potentiated contractions and relaxations in these preparations. These data suggest that a tonically released endocannabinoid is acting at CB₂ receptors and that these receptors are also activated by exogenous CBs, though the effects of WIN55,212-2 were not antagonized by AM630. A slightly different picture emerges from studies in the mouse stomach (Mulè et al., 2007b). Here CB₂ receptors modulate excitatory transmission by reducing contractility, but they are not effective in modulating relaxations and, in these preparations, there was no evidence of tonic activation of CB receptors. Moreover, the effects of WIN55,212-2 were partially antagonized by AM630 and completely blocked by a combination of rimonabant and AM630. Finally, there is recent evidence that CB₂ receptors may have a role in the regulation of intestinal motility as Kurjak et al. (2008) showed that anandamide stimulated VIP release from synaptosomal preparations of the rat ileal myenteric plexus. Further studies that examine the role of CB₂ receptors in enteric neurotransmission are required.

There are few mechanistic studies that describe the action of CB₁ receptors at the cellular level in the ENS, especially in the context of the ongoing neural activity that is important for normal gut function. Boesmans et al. (2009) have begun to address this deficiency using primary cultures of the myenteric plexus. They show that CB₁ receptor antagonists increase spontaneous neural network activity in both excitatory and inhibitory neurons, an effect that was abolished by methanandamide, but curiously not by the mixed CB receptor agonist WIN55,212-2. Inhibition of FAAH was also able to reduce spontaneous neural network activity (Boesmans et al., 2009). These new data suggest that endogenously released endocannabinoids regulate tone in the ENS (Galligan, 2009). However, the study of Boesmans et al. (2009) went further and also showed that CB₁ receptors were able to alter the recycling of neurotransmitter vesicles and mitochondrial transport in the enteric nerve fibres. These results show that CB₁ receptors regulate the probability of vesicle release at the terminals of enteric nerves (Boesmans et al., 2009). Future studies are required to extend these important observations to intact preparations of the ENS and to show whether enteric synapses are under tonic control by the endocannabinoid system. Nevertheless, this study serves to illustrate why patients taking CB₁ receptor antagonists for the treatment of obesity typically display side-effects related to alterations of GI motility (Van Gaal et al., 2005; Addy et al., 2008).

5.5.2. Gastric emptying, intestinal transit and colonic propulsion in vivo

Plant-derived, endogenous and synthetic CB receptor agonists have been shown to reduce gastric emptying (Calignano et al., 1997; Izzo, Mascolo, Capasso, et al., 1999; Landi et al., 2002; Di Marzo et al., 2008; Abalo et al., 2009), upper GI transit (Colombo et al., 1998; Izzo et al., 1999b; Izzo et al., 2000b; Landi et al., 2002; Carai et al., 2006; Izzo et al., 2009b) and colonic propulsion (Pinto et al., 2002) in

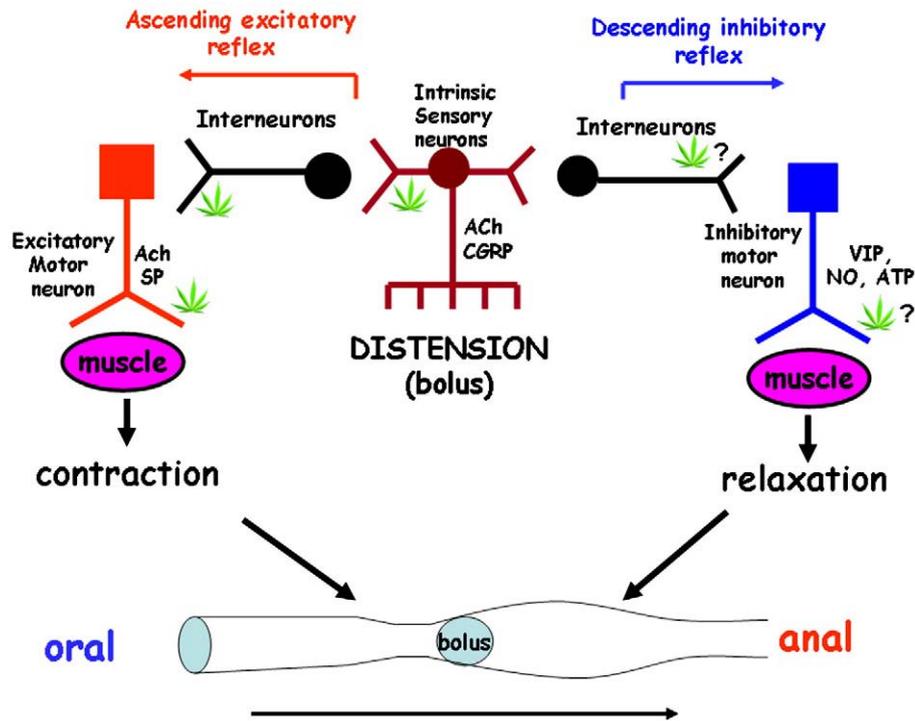


Fig. 1. Sites of action of cannabinoids in the enteric nervous system. Peristalsis, which occurs in response to the radial distension of the intestinal wall, is a coordinated pattern of motor behaviour which occurs in the GI tract and allows the contents to be propelled in an anal direction. The pathways mediating peristalsis involve intrinsic sensory neurons and interneurons, as well as excitatory and inhibitory motor neurons. Acetylcholine (ACh) acting through both muscarinic and nicotinic receptors and tachykinins are excitatory neurotransmitters participating in the peristaltic activity, whereas VIP, nitric oxide (NO) and ATP (or a related purine) act as inhibitory mediators. CB₁ receptors (indicated with the marijuana leaf) immunoreactivity has been identified on intrinsic sensory neurons, ascending neurons and final excitatory motor neurons projecting into longitudinal and circular muscles. Pharmacological evidence suggests that CBs inhibit both the ascending contraction (and concomitant acetylcholine and substance P (SP) release) and descending relaxation (and concomitant VIP release). Additionally, CBs inhibit CGRP release from intrinsic sensory neurons. The question mark indicates that there is pharmacological, but not immunohistochemical, evidence for the presence of the CB₁ receptor (adapted from Aviello et al., 2008b).

rodents. The inhibitory action of CB receptor agonists was counteracted by CB₁, but not CB₂ receptor or TRPV1 antagonists (Izzo et al., 2001a), suggesting a selective involvement of CB₁ receptors. Inhibitors of endocannabinoid inactivation such as FAAH or MGL inhibitors also inhibited GI motility, an effect reduced or abolished by selective CB₁ receptor antagonists or in CB₁ receptor-deficient mice (Capasso et al., 2005; Di Marzo et al., 2008; Duncan et al., 2008b). Furthermore, pharmacological blockade of CB₁ receptors (Colombo et al., 1998; Izzo et al., 2000b; Pinto et al., 2002; Di Marzo et al., 2008) or genetic deletion of the CB₁ receptor (Yuce et al., 2007; Sibaev et al., 2009) exerted prokinetic effects along the GI tract. Collectively, such findings suggest that endocannabinoids are physiologically involved in the regulation of gastric and intestinal motility and that myenteric CB₁ receptors constitute a physiological “brake” along the GI tract in vivo.

Consistent with animal studies, orally administered dronabinol (Δ^9 -THC) reduces gastric emptying in humans (Esfandyari et al., 2006), though in women to a greater extent than men. However, it had no effect on intestinal or colonic transit, possibly due to rapid metabolism (Esfandyari et al., 2006). When taken orally it also decreased postprandial colonic tone and increased compliance of the colon (Esfandyari et al., 2007). Further studies are clearly required, but these results illustrate the importance of doing translational studies that examine the actions of the endocannabinoid system in humans, in order to explore whether there are indeed therapeutic options that might be exploited (see Sanger, 2007, for further discussion).

5.5.3. Motility in pathophysiological states

Both CB₁ and CB₂ receptors have been implicated in the regulation of intestinal motility in pathophysiological states. Sibaev et al. found that inflammation induced by intracolonic dinitrobenzene sulphonic

acid (DNBS) caused spontaneous rhythmic action potentials in smooth muscle cells in CB₁-deficient but not in wild-type mice, suggesting that the altered neuromuscular control mechanisms that contribute to dysmotility are regulated by CB₁ receptors (Sibaev et al., 2006). In the croton oil model of ileitis, CB₁ receptors were found to be over-expressed and CB agonists were consequently more active in reducing transit compared to control mice (Izzo et al., 2001b). In experimental ileus induced by acetic acid in mice, the CB₁ receptor antagonist rimonabant, but not the CB₂ receptor antagonist SR144528, exerted prokinetic effects (Mascolo et al., 2002). On the other hand, enhanced intestinal transit due to lipopolysaccharide (LPS) was reversed by a CB₂ but not by a CB₁ receptor agonist in the rat in vivo (Mathison et al., 2004). Detailed studies in the isolated rat ileum showed that the CB₂ receptor agonist JWH133 did not affect the electrically-evoked contractions under physiological conditions, whereas it was able to reduce the contractile response – and the associated increase in Fos expression in enteric glia and neurons – in mice treated with LPS (Duncan et al., 2008a).

In summary, both CB₁ and CB₂ receptor activation may reduce hypermotility associated with gut inflammation and/or immune activation in rodents. Whether this is true in humans remains to be determined.

5.6. Secretion and ion transport

Fluid secretion is an essential element of digestion, as well as host defence. Furthermore, adequate fluid secretion is required for the normal passage of gut contents along the bowel. In the colon, fluid is absorbed limiting water loss; a failure to absorb water or any situation of excessive secretion leads to diarrhoea. The importance of the endocannabinoid system in the control of secretion is underscored by

the fact that rats given the CB₁ receptor antagonist rimonabant have higher fecal water content than animals treated with vehicle (Izzo et al., 1999b).

Experimental data suggest that CBs and endocannabinoids inhibit the hypersecretion induced by cholera toxin in the mouse small intestine via CB₁ receptor activation (Izzo et al., 2003; Izzo & Capasso, 2006) and delay the onset of castor oil-induced diarrhoea in the rat (Izzo et al., 1999b). Studies monitoring electrolyte movement in muscle-stripped sheets of intestine mounted in Ussing chambers revealed that activation of CB₁ receptors located on submucosal neurons and extrinsic primary afferents in the submucosa reduces ion transport (Tyler et al., 2000; MacNaughton et al., 2004).

5.7. Visceral sensation

CBs have been shown to reduce visceral sensation and pain in a number of experimental models of visceral pain, including the abdominal response to colorectal distension, the acetic acid-induced abdominal stretching and the visceral hyperalgesia due to water avoidance stress.

Both CB₁ and CB₂ receptor agonists have been shown to reduce the degree of visceral sensitivity associated with colorectal distension under basal conditions (Sanson et al., 2006; Fioramonti & Bueno, 2008; Kikuchi et al., 2008; Brusberg et al., 2009; Ravnefjord et al., 2009); this effect was more evident if abdominal hypersensitivity was induced by an inflammatory stimulus (Sanson et al., 2006; Kikuchi et al., 2008). The CB₂ receptor-induced analgesic effect could be due to inhibition of the sensitizing effects of proinflammatory/algic compounds on peripheral endings of visceral afferent nerves. Hillsley et al. (2007) have shown that the CB₂ receptor agonist AM1241 reduces activation of mesenteric primary afferents stimulated by the algic compound bradykinin in wild-type but not in CB₂ receptor-deficient mice (Hillsley et al., 2007). The importance of CB₂ receptors in visceral sensation has been further strengthened by the observation that oral administration of probiotics, which play a role in the clinical management of IBS (Camilleri & Chang, 2008), reduced colorectal distension-induced visceromotor responses in rats in a CB₂ receptor antagonist-sensitive manner (Rousseaux et al., 2007).

Δ⁹-THC and cannabidiol elicited, via CB₁ receptor activation, anti-nociceptive effects in the acetic acid model of visceral nociception (acetic acid-induced abdominal stretching) (Booker et al., 2009). Furthermore, genetic deletion or pharmacological inhibition of FAAH resulted in anti-nociceptive effects, an action that was sensitive to a CB₁, but not a CB₂ receptor antagonist (Naidu et al., 2009). Transgenic mice that expressed FAAH exclusively in the nervous system displayed an anti-nociceptive phenotype, indicating the involvement of peripheral FAAH. In addition, combination of the FAAH inhibitor URB597 and the non-selective cyclooxygenase inhibitor diclofenac yielded synergistic analgesic effects (Naidu et al., 2009).

Visceral hyperalgesia in response to psychological stress (1-h water avoidance) is associated with reciprocal changes in the expression and function of CB₁ (decreased) and TRPV1 (increased) receptors in rat dorsal root ganglion neurons (Hong et al., 2009). These reciprocal changes in CB₁ and TRPV1 receptors were reproduced by treatment of control dorsal root ganglia with anandamide, whose levels are increased following water avoidance stress. Additionally, the CB receptor agonist WIN55,212-2 and the TRPV1 antagonist capsazepine prevented the development of visceral hyperalgesia and blocked the up-regulation of TRPV1. Collectively, such results suggest that CB₁ and TRPV1 receptor pathways may play an important role in stress-induced visceral hyperalgesia (Hong et al., 2009). In a different study, activation of CB₁ receptors was found to reduce the activity of TRPV1 in rat cultured primary sensory neurons (Mahmud et al., 2009). Such action is expected to provide significant pain relief and to reduce visceral hyperactivity in inflammatory conditions (Mahmud et al., 2009).

In summary, direct or indirect CB₁ receptor activation as well as direct CB₂ receptor activation inhibits visceral sensitivity and pain in rodents. The CB₁ receptor-mediated analgesic effect is associated with down-regulation of TRPV1, whilst CB₂ receptor agonist inhibition of visceral pain responses appears to be due to inhibition of the algic responses to bradykinin.

5.8. Inflammation

Anecdotal reports indicate that IBD patients may experience relief by smoking marijuana (Di Marzo & Izzo, 2006). Preclinical experiments in humans have shown increased expression of CB receptors and/or enhanced endocannabinoid levels in intestinal biopsies of patients with gut inflammatory diseases, including ulcerative colitis, Crohn's disease, diverticulitis and coeliac disease (Ligresti et al., 2003; Wright et al., 2005; Guagnini et al., 2006b; D'Argenio et al., 2007).

In vitro studies have highlighted the importance of both CB₁ and CB₂ receptors in modulating inflammatory processes. CBs have been shown to promote epithelial wound healing in a CB₁ receptor-sensitive manner in the human colon (Wright et al., 2005). On the other hand, Ihenetu et al. found that CB₂ receptor activation by CBs exerted an inhibitory effect on interleukin (IL)-8 release in human colonic epithelial cells, which are recognised to exert a major influence on the maintenance of intestinal immune homeostasis (Ihenetu et al., 2003).

Evidence based on well-established models of IBD in rodents [i.e. DNBS, trinitrobenzene sulphonic acid (TNBS) and oil of mustard-induced colitis] indicates that endocannabinoids may limit intestinal inflammation via CB₁ and/or CB₂ receptor activation (Di Marzo & Izzo, 2006; Smid, 2008). Indeed (i) both CB₁ and CB₂ receptor agonists reduced experimental inflammation induced by oil of mustard or TNBS (Kimball et al., 2006; Storr et al., 2009a,b), (ii) genetic ablation of CB₁ receptors, as well as pharmacological treatment with a CB₁ receptor antagonist, rendered mice more sensitive to colitis induced by intracolonic DNBS (Massa et al., 2004); (iii) JWH133 or AM1241, two selective CB₂ receptor agonists, reduced, whilst the CB₂ receptor antagonist AM630 exacerbated, TNBS-induced colitis (Storr et al., 2009a,b); (iv) FAAH-deficient mice, which are expected to have higher levels of anandamide, showed significant protection against intestinal inflammation due to DNBS administration (Massa et al., 2004); (v) inhibitors of anandamide reuptake or enzymatic hydrolysis, which increase intestinal anandamide levels, reduced DNBS-induced colonic inflammation in wild-type, but not in CB₁ or CB₂ receptor-deficient mice (D'Argenio et al., 2006; Storr et al., 2008a,b); (vi) endocannabinoid signalling (i.e. anandamide levels and CB receptor protein expression) increased in the inflamed intestine (Izzo et al., 2001b; Mascolo et al., 2002; McVey et al., 2003; Massa et al., 2004; Wright et al., 2005; D'Argenio et al., 2006; Guagnini et al., 2006b; Kimball et al., 2006; D'Argenio et al., 2007; Borrelli et al., 2009).

Specific studies have yet to investigate the gut immune profile of the endocannabinoid system. CB₁ and CB₂ receptors are found on B cells, natural killer cells and mast cells which are involved in immune surveillance of the gut (Klein et al., 2003; Samson et al., 2003; Klein & Cabral, 2006). Stimulated macrophages, mononuclear cells and dendritic cells show increased production of endocannabinoids (Klein & Cabral, 2006) and LPS stimulates anandamide expression in macrophages (Liu et al., 2003). Suppression of activated macrophages and mast cells and secretion of cytokines such as tumour necrosis factor-α (TNF-α) is directly inhibited by cannabinoids (Bueb et al., 2001; Chang et al., 2001; Facchinetti et al., 2003; Samson et al., 2003; Vannacci et al., 2003; Small-Howard et al., 2005). However, other anti-inflammatory mechanisms may be occurring and should be considered in future studies that extend the relatively preliminary observations made to date.

In conclusion, experiments on isolated epithelial cells and in vivo studies using well-established models of IBD indicate that the endogenous CB system, via CB₁ or CB₂ receptor activation, mediates protective effects in the inflamed gut.

5.9. Cancer

The effect of CBs on intestinal carcinogenesis has been evaluated in colorectal carcinoma epithelial cells as well as in experimental models of colon cancer. Preliminary data on gastric cancer cell lines are also available.

CBs, via CB₁ and possibly CB₂ receptor activation, have been shown to exert apoptotic actions in several colorectal cancer cell lines, including SW480, HCT-15, HT29, Caco-2, HCT116, LS174T and SW620 cells (Ligresti et al., 2003; Greenhough et al., 2007; Cianchi et al., 2008; Wang et al., 2008; Izzo & Camilleri, 2009). The mechanism of CB₁ receptor-mediated apoptotic effects involves: i) inhibition of RAS–MAPK and PI3K–AKT pathways (Greenhough et al., 2007), ii) down-regulation of the anti-apoptotic factor survivin, mediated by a cyclic AMP-dependent protein kinase A signalling pathway (Wang et al., 2008); iii) stimulation of the de novo synthesis of the pro-apoptotic lipid mediator ceramide, which also occurs after CB₂ receptor activation (Cianchi et al., 2008) (see Fig. 2). Preliminary data on the human gastric cancer cell line HCB-27 suggest that anandamide has bimodal effects on cell proliferation, i.e. stimulation at concentrations below 1 μM and inhibition at 10 μM or above (Miyato et al., 2008).

CBs may also exert apoptotic actions via CB receptor-independent mechanisms. Indeed, Patsos et al. found that anandamide induced cell death in cyclooxygenase-2 expressing colorectal tumour cells via production of prostamides (prostaglandin ethanalamides) (Patsos et al., 2005), which can be generated by an action of cyclooxygenase-2 on anandamide, which is a substrate for this enzyme (Woodward et al., 2008); also, Gustafsson et al. have recently found that alpha-tocopherol and the nitric oxide synthase inhibitor L-NAME attenuated CB cytotoxicity in human colorectal cancer Caco-2 cells, suggesting an involvement of oxidative stress (Gustafsson et al., 2009).

CBs might exert a protective effect on colon carcinogenesis also through their ability to inhibit the migration of tumour cells, which is a prerequisite for tumour cell invasion and development of metastases. Indeed, selective CB₁, but not CB₂, receptor agonists have been shown to inhibit the migration of the human colon carcinoma cell line SW480 induced by norepinephrine (Joseph et al., 2004).

Interactions between the endogenous CB system and well-established anti-tumour drugs have been reported. 17β-Estradiol induced CB₁ gene expression in DLD-1, HT-29 and SW620 human colorectal cancer cells (Notarnicola et al., 2008). Anandamide synergistically enhanced the apoptotic action of the anti-tumour drug paclitaxel, possibly through the activation of caspase-3, -8 and -9 in the human gastric cancer cell line HCB-27 (Miyato et al., 2008). Finally, the CB receptor agonist HU210 and the anti-tumour drug 5-fluorouracil produced synergistic anti-proliferative effects in human Caco-2 colorectal cancer cells (Gustafsson et al., 2009).

In vivo, CBs have been evaluated against colon carcinogenesis induced by the carcinogenic substance azoxymethane, by xenografts in nude mice as well as in *Apc* mice. Results suggest that CBs might be protective at different stages of colon cancer progression either directly, through activation of CB₁ or CB₂ receptors, or indirectly, through elevation of endocannabinoid levels, via FAAH inhibition. Indeed i) the FAAH inhibitor AA-5-HT and the CB receptor agonist HU-210 reduced the development of the precancerous lesions induced by the carcinogenic agent azoxymethane in mice (Izzo et al., 2008); ii) pharmacological inhibition or genetic deletion of CB₁ receptors in *Apc* mice resulted in an increase in small intestinal and colonic polyp burden; conversely, the CB₁ receptor agonist R-1 methanandamide reduced the number of tumours in the small intestine and colon (Wang et al., 2008); iii) peritumoural treatment with the CB₂ receptor agonist CB13 reduced the growth of tumours obtained by injection of colorectal carcinoma cells in immunodeficient mice (Cianchi et al., 2008).

Adaptive changes of the endogenous CB system, which include increased endocannabinoid levels, down-regulation of CB₁ and up-regulation of CB₂ receptor expression, have been observed in intestinal biopsies from colon cancer patients (Wang et al., 2008; Cianchi et al., 2008). Changes in DNA methylation and histone modifications of the CB₁ receptor gene *cnr 1*, called epigenetic silencing, are believed to contribute to loss of CB₁ receptor transcription in colon cancer specimens (Wang et al., 2008), which further supports the concept that, in humans, loss of CB₁ receptor expression could be associated with the progression of colorectal cancer (Wang et al., 2008). Additionally, patients with Dukes stages C and D colon cancer had a 2.9 times, and

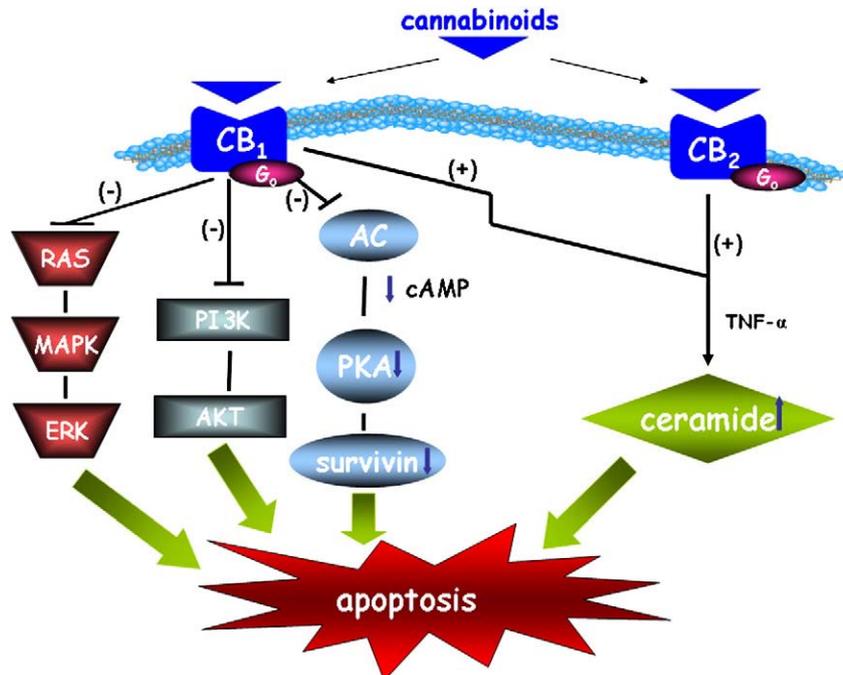


Fig. 2. Intracellular pathways underlying the pro-apoptotic effect of cannabinoids in the gut. Activation of both CB₁ and CB₂ receptors by endogenous, synthetic and plant CBs can induce apoptosis in colon cancer cells. The mechanism of CB₁ receptor-mediated apoptosis involves inhibition of both RAS–MAPK/ERK and PI3K–AKT survival signalling cascade and down-regulation of the anti-apoptotic factor survivin (mediated by a cyclic AMP-dependent protein kinase A signalling pathway). The pro-apoptotic lipid ceramide could be involved in both CB₁ and CB₂ receptor-mediated anti-tumour effects, with TNF-α acting as a link between CB receptor activation and ceramide production.

patients that were lymph node positive had a 2.8 times greater probability of nucleotide changes in the *cnr 1* gene (Bedoya et al., 2009). This is relevant in the light of the observation that a nucleotide change in the *cnr 1* gene exerts a modulating effect on the evolution and outcome of colon cancer patients.

In summary, down-regulation of CB₁ receptors and up-regulation of CB₂ receptors have been observed in intestinal specimens of colon cancer patients. In colorectal carcinoma cell lines, CBs, via activation of CB₁, and possibly, CB₂ receptors and/or via non-CB mediated mechanisms, such as prostamide production, could exert anti-proliferative, anti-metastatic and pro-apoptotic actions. In vivo, CBs might be protective in different stages of colon cancer progression either directly, through activation of CB₁ or CB₂ receptors, or indirectly, through elevation of endocannabinoid levels via FAAH inhibition.

6. Pharmacological interactions with other receptor systems

6.1. Transient receptor potential vanilloid 1 (TRPV1)

The endocannabinoid anandamide can activate TRPV1 receptors, whose distribution on extrinsic primary afferent nerve fibres overlaps with that of CB₁ receptors in the GI tract (see above). There are no studies that directly examine the interactions of these receptor systems in intestinal tissues, but at other sites it has been shown that constitutive activation of the CB₁ receptor influences the ability of TRPV1 to be sensitized. Here it was shown that CB₁ receptor inverse agonists concentration-dependently inhibited capsaicin-induced calcium influx in a cell line and a dose-related inhibition of flinching in mice (Fioravanti et al., 2008). Whether this occurs in the GI tract is not known, but such an interaction is certainly possible given the anatomical overlap in the distribution of these receptors. Whether there is a direct interaction or not, reciprocal changes in the expression of CB₁ and TRPV1 receptors contribute to stress-induced visceromotor reflexes to colonic distension (Hong et al., 2009). Stressed rats had increased levels of anandamide in their dorsal root ganglia, which were associated with the reduced CB₁ receptor expression and increased TRPV1 expression. Interestingly, treatment of control dorsal root ganglia with anandamide in vitro mimicked the effects on receptor expression, suggesting that the endocannabinoids act not only as ligands but also as auto-regulatory molecules for their cognate receptors.

Though endocannabinoids are anti-inflammatory in the colon (see above), McVey et al. (2003) show that anandamide activates TRPV1 receptors to elicit a neurogenic inflammation in the ileum. Neither CB₁ nor CB₂ receptor antagonists blocked the actions of these endocannabinoids, whereas their effects were blocked by the TRPV1 antagonist capsazepine and a tachykinin NK1 receptor antagonist, suggesting that activation of TRPV1 led to the release of substance P or a related tachykinin from primary afferent nerves in the intestine. This mechanism has also been proposed to account for the enhanced acetylcholine release in the guinea pig ileum (Mang et al., 2001). It should also be noted that anandamide has been shown to regulate the release of CGRP from primary afferent nerves in a manner that was dependent on the state of activity of the CB₁ receptor, since this was modulated by rimonabant (Ahluwalia et al., 2003). Anandamide is also able to depolarize vagal nerve fibres although this is through non-CB₁/CB₂ receptor mechanisms (Kagaya et al., 1992).

Primary afferent nerves are clearly involved in neurogenic inflammation in the GI tract and their activation also leads to states of chronic pain (Holzer, 2008a). One important mediator of inflammation and pain is the neurotrophin nerve growth factor (NGF). NGF levels appear to modulate TRPV1 receptor activation by anandamide (Evans et al., 2007), suggesting that NGF may regulate the crosstalk between these receptors. The level of NGF regulates the proportion of primary afferent neurons responsive to anandamide. At low NGF levels, CB₁ receptor activation appears to inhibit TRPV1 activation; blocking the CB₁ receptor with rimonabant increases the proportion of

neurons activated by capsaicin. In contrast, at higher NGF levels, TRPV1 expression is enhanced and the proportion of neurons responding to capsaicin is greater. CB₁ receptor activation now leads to enhanced calcium influx in these cells. Further investigation of these interactions in identified neurons that project to the gut is clearly warranted.

6.2. Kappa-opioid receptors

There is a substantial overlap between the activity of CBs and opioids in the GI tract. Nevertheless direct evidence for interactions between these receptor systems is not well established despite evidence for co-localization of kappa-opioid, delta-opioid and CB₁ receptors on myenteric neurons (Kulkarni-Narla & Brown, 2000). Recently, however, Capasso et al. (2008b) investigated kappa-opioid/CB interactions using the natural kappa-opioid receptor ligand Salvinorin A. They showed that in croton oil-induced ileitis, but not in healthy controls, the CB₁ receptor antagonist rimonabant was able to suppress kappa-opioid receptor agonist mediated hypomotility. Extending these novel observations, Fichna et al. (2009) found that in the isolated colon, there are interactions between kappa-opioid receptors and CB₂ receptors. They showed that veratridine-stimulated secretion from the healthy colon was inhibited by Salvinorin A. The effects of Salvinorin A (which is not a CB₂ receptor ligand) were blocked by the CB₂ receptor antagonist AM630 as well as a kappa-opioid receptor antagonist (Fichna et al., 2009). Whether these interactions occur in vivo is not known. These studies open the possibility that both in health and disease there are interactions between opioid and CB receptors, and that therapies combining mixtures of appropriate agonists (or antagonists) of these receptor systems might prove to be of value in the treatment of certain GI disorders.

6.3. Other receptor systems

Interactions with other receptor systems in the gut are likely, but have not been fully characterized. For example, Mang et al. (2001) demonstrated that whilst anandamide regulated basal acetylcholine release through TRPV1 receptors, electrically-evoked release was inhibited by anandamide, but this was neither mediated by CB or TRPV1 receptors. Similarly Kojima et al. (2002) demonstrated that 2-AG and anandamide caused a tetrodotoxin-sensitive contraction of the longitudinal muscle of the guinea pig colon that was not blocked by CB receptor or TRPV1 antagonists, but was reduced by a lipoxygenase inhibitor. Along similar lines, cholinergic contractions of the human colon were inhibited by 2-AG and anandamide through a mechanism that does not involve CB receptors (Smid et al., 2007). Capasso et al. (2008a) showed that cannabidiol (CBD) inhibited acetylcholine-induced contractions in the isolated mouse ileum, which is suggestive of a direct action on gut smooth muscle. Since the receptor involved was not determined it is difficult to speculate what the mechanism might be, but CBD is unlikely to be a muscarinic antagonist since it also blocked the actions of prostaglandin F_{2α} (Capasso et al., 2008a).

One better characterized system where there are interactions of various receptor systems is in the nodose ganglion that supplies the vagal afferent innervation of the gut. Low levels of CB₁ receptor expression are generally observed in the nodose ganglion of fed rats (Burdyga et al., 2004). However, CB₁ receptor expression is increased during fasting, notably in vagal afferent neurons innervating the gut that also express cholecystokinin (CCK)₁ receptors whose expression is not altered by fasting. The down-regulation of CB₁ receptor expression by feeding is mediated by CCK₁ receptors, since it is abolished by the CCK₁ receptor antagonist lorglumide (Burdyga et al., 2004). These results illustrate an interesting reciprocal action whereby CCK, a satiety factor, blocks the action of endocannabinoids, which are orexigenic. The system is, however, more sophisticated. Vagal afferent neurons also express receptors for the orexigenic gastric peptide ghrelin (Burdyga et al., 2006). Ghrelin receptor activation prevents the down-regulation

of CB₁ receptors by CCK. Thus the vagus serves as a gatekeeper for the complex interactions that provide a balance between orexigenic and anorexigenic signalling from the GI tract.

Finally, evidence for a role of CBs in the modulation of purinergic transmission has been recently provided. It was found that the inhibitory tetrodotoxin- and atropine-sensitive effect of the CB receptor agonist *N*-(2-chloroethyl)5,8,11,14-eicosaetraenamide (ACEA) on the spontaneous contractions of the isolated mouse ileum was counteracted by a selective P2X receptor antagonist (Baldassano et al., 2009). In addition, ACEA, atropine and tetrodotoxin inhibited the contractions induced in the ileum by the P2X purinoceptor agonist α,β -methylene-ATP. Collectively such results suggest that CBs inhibit endogenous purinergic effects, mediated by P2X receptors, on cholinergic neurons (Baldassano et al., 2009).

7. Gastrointestinal signs of tolerance

Tolerance to the actions of Δ^9 -THC was reported over thirty years ago (Anderson et al., 1975). Repeated dosing over 4 days reduced the inhibitory effects of Δ^9 -THC on intestinal transit. This reduced efficacy persisted for more than two weeks after the last administration of the drug. In contrast, when anandamide was repeatedly given over a two week period and stress-induced defaecation was measured, it was found that there was no tolerance to a subsequent administration of this endocannabinoid (Fride, 1995). However, animals treated with anandamide displayed tolerance to Δ^9 -THC. This assay is not strictly one of motility and hence the site of tolerance, or lack of tolerance, could not be clearly identified. Using a novel radiological method, Abalo et al. (2009) directly assessed GI motility and studied tolerance to repeated administration over two weeks of the mixed CB₁/CB₂ receptor agonist WIN55,212-2. They showed that tolerance did not develop for the delayed gastric emptying that is produced by administration of WIN55,212-2. A slight degree of tolerance developed in the small intestinal motility, whereas in the large bowel there was a clear indication of tolerance (Abalo et al., 2009). A week after the last administration of WIN55,212-2 there were no persistent effects. Whether the differences in these 3 studies are due to the metabolism of the different CB receptor agonists is not clear. However, in general the GI tract in vivo does appear to be relatively resistant to tolerance to CB receptor agonists.

As noted above, the CB₁ receptor inverse agonist/antagonist rimonabant increases GI motility. Tolerance to its actions rapidly occurs in mice upon repeated administration (Carai et al., 2004). Clinical trials revealed that rimonabant has GI effects consisting of diarrhoea and nausea (Van Gaal et al., 2005; Pi-Sunyer et al., 2006; Van Gaal et al., 2008). These effects are dose-related and also appear to wane over time, suggestive of tolerance.

In vitro, where the exact site of action can be established, Pertwee demonstrated that the inhibitory effects of CP55,940 on electrically-evoked contractions of the longitudinal muscle were reduced in animals treated with large doses of Δ^9 -THC for two weeks, suggesting tolerance at the level of the myenteric plexus (Pertwee, 2001). This was confirmed in isolated preparations of guinea pig longitudinal muscle-myenteric plexus and human small intestinal segments (Guagnini et al., 2006a). Here WIN55,212-2 was used as the agonist and by using rimonabant these authors showed that the tolerance occurred at the CB₁ receptor. This finding confirmed earlier work by Basilico et al. (1999). They also showed evidence of cross-tolerance to morphine in their studies.

Further mechanistic studies that examine the site and nature of the tolerance and cross-tolerance are required to understand the therapeutic consequences of these effects if endocannabinoid-based CB agonists are developed for clinical use (Storr et al., 2008a,b).

8. Anandamide-related acylethanolamides (AEs): effects in the digestive tract

AEs are an important family of lipid-signalling molecules widely distributed in plants, invertebrates and mammals (Matias et al., 2007;

Borrelli & Izzo, 2009; Hansen & Diep, 2009). The best-studied AEs are the endocannabinoid anandamide and non-endocannabinoid compounds such as PEA and OEA, both of which are also substrates for FAAH. The molecular targets of PEA and OEA, which have been identified in the GI tract, include TRPV1, activated by OEA, the nuclear receptor PPAR α (activated by OEA and, to a lesser extent, by PEA) and the orphan G-coupled receptors GPR119 (activated by OEA) and GPR55 (activated by PEA and, with lower potency, by OEA) (Brown, 2007; Capasso & Izzo, 2008; Overton et al., 2008; Thabuis et al., 2008; Borrelli & Izzo, 2009). Both PEA and OEA are synthesized in the digestive tract and their levels may change in response to inflammation, diet or food deprivation (Borrelli & Izzo, 2009). Thus, i) PEA increases in intestinal biopsies of patients with coeliac disease as well as in the experimental model of coeliac-like disease induced in rats by methotrexate (D'Argenio et al., 2007); ii) OEA increases in the stomach of diet-induced obese mice (Aviello et al., 2008a); iii) OEA, which increases in the intestine in response to food intake, activates PPAR α to evoke satiety (Fu et al., 2003; Lo Verme et al., 2005).

Pharmacological studies have shown that PEA and OEA reduced GI motility (Capasso et al., 2001; Capasso et al., 2005; Aviello et al., 2008a; Cluny et al., 2009). More in-depth investigations revealed that OEA inhibited gastric emptying and small intestinal transit in a PPAR α and CB receptor-independent manner (Aviello et al., 2008a; Cluny et al., 2009). Importantly, OEA blocked stress-induced accelerated small intestinal transit at a dose that had no effect on physiological transit (Aviello et al., 2008a; Cluny et al., 2009). Lastly, OEA has been shown to reduce visceral pain in a PPAR α -insensitive manner (Suardíaz et al., 2007) and enhance fatty acid uptake in enterocytes (Yang et al., 2007).

These studies point to the importance of considering all potential products of FAAH inhibition when considering the consequences of this approach as a therapy in the gut. Since potentially all AEs will be altered by blocking FAAH, efforts are required to establish what are the dominant actions of AEs and how these may change in the disease.

9. Plant non-psychotropic cannabinoids: effects in the digestive tract

The plant *Cannabis* contains not only Δ^9 -THC, but also a number of CBs with weak or no psychoactivity, which therapeutically might be even more promising than Δ^9 -THC (Pertwee, 2008; Izzo et al., 2009a; Scuderi et al., 2009). These include cannabidiol (CBD), Δ^9 -tetrahydrocannabinavarin (Δ^9 -THCV, a CB₁ receptor antagonist), cannabigerol (CBG), cannabichromene, cannabidivarin (CBDV) and acidic CBs such as Δ^9 -tetrahydrocannabinolic acid (Δ^9 -THCA) and cannabidiolic acid (CBDA) (Izzo et al., 2009a).

Δ^9 -THC, CBD, CBG and cannabichromene, but not Δ^9 -THCA or CBDA, were shown to exert anti-proliferative actions in human colorectal carcinoma (Caco-2) cells and in gastric adenocarcinoma cells with an IC₅₀ in the 7.5–21.5 μ M range. CBD and CBG were found to be the most active compounds investigated (Ligresti et al., 2006). In vivo, the quinone of CBD (named HU-331) was more active but less toxic than doxorubicin in a colon carcinoma model induced by tumour xenograft injection in nude mice (Kogan et al., 2007).

CBD was effective in animal models of anticipatory nausea and vomiting (e.g., conditioned gaping in rats and conditioned retching reactions elicited in the house musk shrew), suggesting a potential in the treatment of chemotherapy-induced nausea (Parker et al., 2002; Parker & Mechoulam, 2003; Parker et al., 2004, 2006; Rock et al., 2008). CBD also induced a small non-significant reduction of food intake and weight gain in mice (Riedel et al., 2009). By contrast, Δ^9 -THCV shares the ability of synthetic CB₁ receptor antagonists to reduce food intake and body weight in mice (Riedel et al., 2009).

CBD has also been shown to be protective in the murine model of colitis induced by DNBS (Borrelli et al., 2009). The effect of CBD was associated with down-regulation of inducible nitric oxide synthase expression and modulation of cytokine (IL-1 β and IL-10) levels. Studies on intestinal epithelial cells suggest that CBD prevents oxidative stress,

which may be one of the underlying factors leading to mucosal protection in vivo (Borrelli et al., 2009). In a different study, CBD did not affect motility in control mice, but normalized inflammation-induced hypermotility in a CB₁ receptor antagonist and FAAH-inhibitor sensitive manner (Capasso et al., 2008a). These data suggest that CBD may inhibit motility via indirect activation of CB₁ receptors. Results along the same line were reported by De Filippis et al., who demonstrated that CBD inhibited intestinal transit in a CB₁ receptor-sensitive manner when tested in mice with sepsis-induced motility disturbances and also inhibited FAAH expression in the inflamed, but not healthy, intestine (De Filippis et al., 2008).

Lastly, an early review article mentioned that Δ^9 -THCA affected the isolated rabbit intestine and exhibited a non-surmountable antagonism of contractions of the isolated rat ileum induced by different spasmogenic agents (Turner et al., 1980).

In summary, amongst the non-psychotropic plant CBs, CBD has been most thoroughly investigated. Results suggest that CBD exerts an anti-proliferative action in vitro and beneficial actions in counter-acting nausea as well as hypermotility and mucosal inflammation associated with experimental IBD. In addition, Δ^9 -THCV represents a novel CB₁ receptor antagonist which has potentially useful actions for the treatment of obesity.

10. Genetic variation, gastrointestinal function and pharmacogenetic considerations

Given the significant functional activity of CBs in the gut, alterations of the endocannabinoid system through genetic variations may be postulated to underlie various GI disorders. Few studies have yet examined the genetics of the endocannabinoid system in the gut, because most of the polymorphisms in CB genes are only recent discoveries and epigenetic changes have not been considered to any great extent until lately. Exciting findings are now emerging.

Gene expression is frequently controlled by the extent of methylation of the gene promoter (Suzuki & Bird, 2008). In colorectal cancer, the expression of the CB₁ receptor was markedly reduced and this was found to be due to hypermethylation on CpG islands of DNA in the CB₁ receptor gene promoter (Wang et al., 2008). The hypermethylation reduces the activity of the promoter and hence gene expression is reduced. In contrast, no changes were observed in the expression of CB₂ receptors in colorectal tumours. A number of single nucleotide polymorphisms (SNPs) have been described in the CB₁ receptor gene. These have been associated with anorexia nervosa, bulimia and obesity (e.g. Benzinou et al., 2008; Monteleone et al., 2009). The G1359A mutation has recently been shown to occur in colorectal cancer (Bedoya et al., 2009). This SNP was commonly found in specimens from advanced disease and was associated with greater risk of advanced disease, metastases and shorter survival time. Given the anti-proliferative activity of endocannabinoids noted above this is an important finding, and naturally needs to be considered if endocannabinoid-based therapeutic strategies are developed for the treatment for colon cancer. Genetic and epigenetic changes in the endocannabinoid system have not been examined in other GI disorders to date.

A SNP in the FAAH gene (C385A) was shown to result in enhanced sensitivity to proteolytic degradation of FAAH (Sipe et al., 2002). This gene mutation was shown to be more common in patients with functional GI disorders (Camilleri et al., 2008). More specifically, it was associated with changes in colonic transit time in distinct subtypes of these patients. The FAAH SNP was particularly significantly associated with GI symptoms in patients with diarrhoea-predominant and mixed type (diarrhoea and constipation) irritable bowel syndrome and with accelerated colonic transit in patients with diarrhoea-predominant irritable bowel syndrome. This observation at first glance appears to be at odds with expectations. It may be hypothesized that in subjects homozygous for the FAAH SNP, endocannabinoid metabolism would be impaired and degradation of endocannabinoids (and other FAAH

substrates) reduced, which might result in increased local levels, which would be expected to reduce motility. However, the functional alterations caused by the FAAH SNP remain to be fully characterized, limiting further mechanistic interpretation of these data.

The SNP in the FAAH gene has also been analysed with regard to intestinal inflammation in patients with IBD. No significant differences were observed in the frequency of the SNP in Crohn's disease or ulcerative colitis compared to controls (Storr et al., 2008a,b, 2009a,b). However, Crohn's disease patients homozygous for this SNP were more likely to have fistulising disease, which is of greater severity, as well as systemic manifestations of their disease. Thus this SNP may be a disease modifier rather than something that directly leads to disease in susceptible patients.

11. Clinical potential of cannabinoids in gut diseases

Throughout the article we have emphasized the potential of CB-based therapies and discussed some of their limitations. Historically, the major limitation of *Cannabis* or its derivatives for the treatment of GI disorders were the psychotropic side-effects of these agents. These not only limit the doses that can be used, but also their acceptability to a larger patient population. The adverse psychotropic side-effects of CB₁ receptor antagonists likewise have made it unlikely that this class of drug, whilst effective, will be widely used.

There are, however, many other approaches that could be considered that would harness the positive benefits of the CB system: notably utilizing the on-demand nature of endocannabinoid release. As we have shown, the endocannabinoid system is widely distributed throughout the gut, with regional variation and specific regional or organ-specific actions. It is involved in the regulation of food intake, nausea and emesis, gastric secretion and gastroprotection, GI motility, ion transport, visceral sensation, intestinal inflammation and cell proliferation. Many of these processes are subject to disturbances in disease and various animal or preclinical models have shown that modifying the endocannabinoid system can have beneficial effects. In some cases, e.g. nausea, there are few effective alternative therapies, but in other cases, drugs that enhance actions at, or block CB receptors, may be useful adjunct therapies. There is an extensive patent literature that would suggest that biotechnology or pharmaceutical companies are aware of this potential. Thus whilst pathophysiological alterations in the endocannabinoid system may not underlie diseases of the gut, it may be an important disease modifier, with therapeutic potential. This potential will need to be balanced by pharmacogenomic considerations in some cases, e.g. colon cancer.

Drug development focused on the GI tract also seems to be warranted. Disorders of the GI tract are widespread and costly in terms of the health of societies as well as in economic terms. Importantly, there are extensive unmet medical needs for therapies aimed at, for example, colon cancer, irritable bowel syndrome, IBD and many other conditions where the GI tract is affected primarily or as a secondary consequence of systemic disease. There are also opportunities based on our knowledge of the endocannabinoid system to utilize this for benefit, whether it be to reduce inflammation, speed up or slow down motility, reduce cancer cell proliferation or to suppress nausea and emesis. The missing link are drugs with few, if any, central actions. These may take the form of peripherally restricted receptor agonists (Dziadulewicz et al., 2007) or antagonists (Kunos et al., 2009; Lo Verme et al., 2009), or inhibitors of FAAH or MGL whose actions are restricted by virtue of the limited extent of activation of the endocannabinoid system when disease is restricted to the gut. Neutral antagonists (Janero & Makriyannis, 2009) and CB₂ receptor ligands may also offer therapeutic potential for GI diseases and should be further pursued.

12. Conclusions and future directions

The concept of the endocannabinoid system was outlined a mere 14 years ago (Di Marzo & Fontana, 1995). Since then, enormous progress

has been made in defining the elements of this system and how they adapt in the gut to various conditions (see Tables 1 and 2). The endocannabinoid system is an important regulatory system in the GI tract, working in the control of both digestion and host defence; the two major functions of the gut. CB mechanisms have significant potential in GI disease. As the pharmacology of CB mechanisms is increasingly understood, and more selective peripherally or locally acting agents targeting CB₁ and/or CB₂ receptors, or for the activation of endogenous CBs, are developed, there is considerable promise for the treatment of disorders of the gut. Care will need to be taken to fully understand the actions of specific drugs given the disappointment following the withdrawal of rimonabant. Central actions are predictable and it will be essential for future drug development programs to screen any potential class of CB medications for psychotropic potential. With recently emerging data on genetic mutations in elements of the endocannabinoid system, some treatment approaches may have to be tailored to specific subgroups of patients. That said, the potential of this system seems to warrant further investment, both in academia and industry, in order to fully develop it as a therapeutic target in the treatment of GI disorders.

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